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Cortisol response mediates the effect of post-reactivation stress exposure on contextualization of emotional memories

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Abstract

Retrieval of traumatic experiences is often accompanied by strong feelings of distress. Here, we examined in healthy participants whether post-reactivation stress experience affects the context-dependency of emotional memory. First, participants studied words from two distinctive emotional categories (i.e., war and disease) presented against a category-related background picture. One day later, participants returned to the lab and received a reminder of the words of one emotional category followed by exposure to a stress task (Stress group, $n = 22$) or a control task (Control group, $n = 24$). Six days later, memory contextualization was tested using a word stem completion task. Half of the word stems were presented against the encoding context (i.e., congruent context) and the other half of the word stems were presented against the other context (i.e., incongruent context). The results showed that participants recalled more words in the congruent context than in the incongruent context. Interestingly, cortisol mediated the effect of stress exposure on memory contextualization. The stronger the post-reactivation cortisol response, the more memory performance relied on the contextual embedding of the words. Taken together, the current findings suggest that a moderate cortisol response after memory reactivation might serve an adaptive function in preventing generalization of emotional memories over contexts.

Key words: Stress, cortisol, context, reconsolidation, declarative memory, Maastricht Acute Stress Test

1. Introduction

Threatening situations tend to be very well remembered. The ability to vividly remember contextual and specific cues that predict future catastrophes is highly adaptive. Accordingly, memories are generally better retrieved in their original encoding context than in unrelated situations (i.e., memory contextualization, Godden and Baddeley, 1975). When emotional memories generalize over contexts, they may become maladaptive and result in a disproportionate amount of fear and anxiety. Indeed, patients with post-traumatic stress disorder (PTSD) are hindered by superior memory for a traumatic event along with a reduced capacity to associate trauma-related cues to the trauma situation (Liberzon and Sripada, 2007). As a result, the trauma memory is retrieved and relived frequently, which causes an enormous amount of stress. The effect of this post-retrieval stress on emotional memory and specifically on memory contextualization is still largely unknown. Here, we address this issue by examining the role of post-retrieval stress on memory contextualization in healthy participants.

Upon retrieval, a memory trace enters a labile phase followed by a protein synthesis dependent restabilization phase, in which the memory trace is sensitive to change (Nader et al., 2000; Sara, 2000). The alleged functional role of this two-phased reconsolidation process is to keep our memories up to date by either altering their strength (Nader et al., 2000; Soeter and Kindt, 2010, 2011, 2012b) or incorporating new information into a memory trace (e.g., Forcato et al., 2007; Hubbach et al., 2007). A prerequisite to enter the reconsolidation window is that something can be learned during memory retrieval (e.g., Lee, 2009; Sevenster et al., 2013).

A naturalistic event that can affect memory reconsolidation is the experience of a stressor. Stress exposure activates the autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal (HPA) axis, which eventually results in the release of catecholamines ((nor)adrenaline) and glucocorticoids (GCs). Previous studies in animals and humans show a complex interaction between stress and memory reconsolidation, in the sense that stress exposure or the administration of stress hormones can either *strengthen* memory reconsolidation (Frenkel et al., 2005; Cocoz et al., 2011;

Gazarini et al., 2013; Bos et al., 2014) or *disrupt* memory reconsolidation (e.g., Tronel and Alberini, 2007; Maroun and Akirav, 2008; Zhao et al., 2009; Schwabe and Wolf, 2010). These apparently contradictory results may be explained by differences in experimental set-up, type of memory and the strength of the stress response.

It has been suggested that context can play an important role in triggering memory reconsolidation (Forcato et al., 2007; Hupbach et al., 2008; Forcato et al., 2009, 2010), but whether the context-dependency of memory is itself affected during reconsolidation is still unknown. The hippocampus is the key area for binding together multiple elements of an experience into a conjunctive representation (O'Reilly and Rudy, 2001) and for the contextual embedding of memories (e.g., Davachi, 2006). The hippocampus is also highly sensitive to stress hormones (Joëls and Baram, 2009). Previous studies on memory *consolidation* have shown that stress can affect memory contextualization in humans (van Ast et al., 2013; van Ast et al., 2014), which is explained by the high sensitivity of the hippocampus to stress hormones (Joëls and Baram, 2009). The hippocampus is also involved in the process of reconsolidation (Debiec et al., 2002), specifically for context-specific memories (e.g., Winocur et al., 2009). Recent studies in humans and animals suggest that manipulations during the reconsolidation window not only affect the strength of the memory trace itself, but may affect the context-dependency of this memory trace as well (Winocur et al., 2009; Soeter and Kindt, 2012a, 2012b; Gazarini et al., 2013). We showed that pharmacological blockade of the noradrenergic system during the reconsolidation window diminished the subsequent fear response and that this fear-reducing effect generalized to a novel context (i.e. background) (Soeter and Kindt, 2012a). Furthermore, research in animals showed that pharmacological enhancement of the noradrenergic system during the reconsolidation window augmented the expression of fear, which generalized over contexts (Gazarini et al., 2013).

Here, we examined the role of post-reactivation stress exposure on the contextual dependency of emotional memories. In a mixed design, participants learned words from two distinctive emotional categories (i.e., war or disease) against a related background picture (i.e., war

scene or hospital corridor, see Figure 1). Approximately 24 h later, participants returned to the lab and were briefly reminded of the words of one emotional category. This procedure was intended to create a within-subject comparison between reactivated and non-reactivated words. Directly after the reminder procedure, half of the participants were confronted with a stress situation (Maastricht Acute Stress Test; MAST) whereas the other half of the participants received a non-stressful control task. Six days later participants returned to the lab and underwent a surprise word stem completion memory test wherein half of the word stems were presented against the original encoding context (i.e., congruent context) and half of the word stems were presented against the other context (i.e., incongruent context). Thus, in the congruent context condition the contextual information of the encoding situation was present, whereas in the incongruent context condition the contextual information was unrelated to the word to be generated. The difference in memory performance between the congruent and incongruent context provides an index for the contextual dependency of memory. We hypothesized that post-reactivation stress exposure would affect the contextual-dependency of declarative memory in humans. We left the direction of the memory contextualization effect open, given that we could argue either way. From a clinical point of view, we might expect that post-reactivation stress would *impair* the contextual-dependency of memories, whereas based on experimental observation we might expect that post-reactivation stress would *enhance* the contextual-dependency of memory (Winocur et al., 2009; van Ast et al., 2014).

2. Methods

2.1. Participants

Fifty-one healthy students from the University of Amsterdam (23 men and 28 women), ranging in age between 18 and 30 years ($M = 21.84$, $SD = 2.79$) participated in the study. Exclusion criteria were: a neurological or psychiatric condition, blood pressure (BP) $> 140/90$, Beck Depression Inventory (BDI) score > 18 (Beck et al., 1996), taking medication known to influence the HPA-axis and taking drugs on a regular basis, screened with the drug use disorder identification test (DUDIT, Berman et al., 2004).

All female participants used oral contraceptives. Participants received either course credits or a small amount of money (€30,-) for their participation. The study was approved by the local ethical committee of the University of Amsterdam and informed consent was obtained from all participants.

2.2. Stress and control manipulation

Psychosocial stress was induced with the Maastricht Acute Stress Test (MAST, Smeets et al., 2012). The MAST is an effective procedure to elicit subjective, autonomic and glucocorticoid stress responses. The procedure consists of a 5-min preparation period and a 10-min stress phase in which participants are required to immerse their hand in ice-cold water ($M=1.0^{\circ}\text{C} \pm SD=.34$) alternated with mental arithmetic challenges (i.e., counting backwards). The preparation period consists of detailed instructions about the procedure of the MAST on the computer screen. The MAST procedure was administered by another female experimenter. Participants were instructed to look straight in the video camera during the entire test and were explained that the computer signaled the start and the end of the hand immersion and mental arithmetic challenges. During the mental arithmetic test, participants received negative performance feedback from the experimenter concerning accuracy and/or speed of the calculations. In total, participants engaged in 5 hand immersion trials (i.e., duration between 60 and 90 sec) and 4 different arithmetic trials (i.e., duration between 45 and 90 sec).

The control task was equivalent to the MAST, except that the stressful elements were removed (Smeets et al., 2012). Participants were asked to immerse their hand in warm water ($M=35.6^{\circ}\text{C} \pm SD=3.54$) and the mental arithmetic test was replaced by counting aloud from 1 to 25 at their own pace and to start again at 1 when having reached 25. The experimenter was in the room, but did not provide any feedback on performance.

- Insert Figure 1 around here -

2.3. Declarative memory task

2.3.1. Encoding (day 1)

During the encoding phase, participants were randomly shown 48 words on a small grey rectangle against a background picture (see Figure 1). To create two distinctive memory traces, words from two different emotional categories (i.e., 24 war-related words and 24 disease-related words) were presented against a thematic related background picture. All stimuli were presented on a 24" inch computer screen (1920 x 1080 pixels). For stimulus presentation, we used the software package Presentation (Neurobehavioural Systems Inc, www.neurobs.com). The words from the two categories were presented intermixed, with the restriction that no more than three words of the same category were presented in a row. The words were chosen from a recently validated data set (Moors et al., 2012, see supplementary material) with each word having a unique stem (i.e., first 2 letters). Based on the data of Moors et al. (2012), the words of the two emotional categories did not differ in terms of valence (war: $M=2.66$, $SD=.87$; disease: $M=2.53$, $SD=.86$), arousal (war: $M=4.34$, $SD=.99$; disease: $M=4.06$, $SD=.59$), familiarity (log-transformed scores, war: $M=.88$, $SD=.70$; disease: $M=.67$, $SD=.74$) or word length (war: $M=6.79$, $SD=1.79$; disease: $M=6.67$, $SD=1.58$) (all $F_s < 1.45$). At the first trial, participants were instructed to rate the presented words on the dimensions of valence and arousal using self-assessment manikins (SAM; Hodes et al., 1985, Bradley and Lang, 1994). Additionally, participants were asked to focus on the word-context combination. After the first presentation of the words, participants were confronted with two free recall tasks. In counterbalanced order, participants were asked to recall as many words they could remember that were presented on the war-related background context and on the disease-related background context within 2 min. After a 1-min resting period, participants were shown the word-context combinations for a second time. Word order was the same at the second presentation to stimulate the creation of a memory trace of each word list. The second time, participants were explicitly instructed to learn the word-context combinations. Afterwards, the two free recall tasks were presented again (counterbalanced).

2.3.2.Memory reactivation (day 2)

At the second session, memory for one emotional category was reactivated by presenting the free recall task related to these words. Half of the participants received the war-related background, whereas the other half of the participants received the disease-related background. Within each experimental group (stress versus control), participants were randomly assigned to the war or disease-related reactivation condition. Participants were explicitly instructed to recall the words related to the presented context (i.e., background picture) and to perform the free recall task within 2 min. The instruction for the free recall task was similar to the instruction that they received the previous day. Yet, as soon as participants started to type in their response, the free recall task ended abruptly. Thus, participants were not allowed to type in the words they remembered. This procedure was similar to our previous study (Bos et al., 2014) and was used to induce a prediction error, which is a discrepancy between what was expected and what actually occurred (Pedreira et al., 2004; Forcato et al., 2007), which is considered crucial for triggering memory reconsolidation.

2.3.3.Memory test (day 8)

Memory performance was assessed 6 days after memory reactivation with a cued recall task (i.e., word stem completion) and a recognition task (not reported here)¹. In the cued recall task, participants were presented with the first two letters of the study words. The word stems were shown in a grey rectangle against a background picture. To test contextualization of memory, half of the words were presented against their original encoding context (i.e., congruent context), whereas the other half of the words were presented against the other context (i.e., incongruent context). Crucially, the words were randomly assigned to the congruent and incongruent context, with the restriction that half of the recalled words on day 1 were presented in the congruent context and half of the words were presented in the incongruent context for each word category separately. This semi-random assignment of the words to the congruent and incongruent context is necessary to ensure that contextualization effects are not due to encoding effects. Participants were asked to use

¹ Due to procedural and programming errors in the recognition task, this task will not be presented here.

the stem (i.e., 2 letters) as memory cue to complete the word by typing in the remaining letters. Furthermore, they were instructed that the background picture could be the same as on day 1, but not necessarily so. If the participants could not remember the word, they were asked to type in “..”. The task was self-paced.

2.4. Physiological and subjective measurements

2.4.1. Saliva collection

Saliva samples were obtained using cotton salivettes (Sarstedt, Nümbrecht, Germany) at 5 different time points (see Figure 2). For each collection, participants were instructed to place the salivette in their mouth for at least 1 min and allowed to lightly chew on it. The saliva samples were stored at -30 °C until biochemical analysis were performed. Free salivary cortisol concentrations were determined using an enzyme-linked immunoassay (Cortisol Elisa, RE52611; IBL international, Hamburg, Germany). Analyses were carried out according to the manufacturers’ instructions (intra-assay variability 1.2 -6.7%). Salivary alpha-amylase concentrations were determined with a commercially available enzymatic assay (Alpha-Amylase saliva assay, RE80111; IBL international, Hamburg, Germany), following the instructions outlined by the kit manufacturer (IBL) (intra-assay variability 0.03 – 5.23%). Cortisol and alpha-amylase concentrations were assayed to test whether the stress manipulation succeeded. Given the difference in expected peak level between cortisol (peak level 20 min after stress manipulation) and alpha-amylase (peak level directly after stress manipulation), we analyzed S1 to S4 for cortisol and S1 to S3 for alpha-amylase.

- Insert Figure 2 around here -

2.4.2. Blood Pressure and Heart Rate

Blood pressure (BP) and heart rate were measured with a fully automated upper-arm oscillometric BP monitoring device (Omron M2). BP and HR were assessed at 7 different time point (see Figure 2).

2.4.3. Subjective experience of stress

To assess to what extent participants experienced the MAST as stressful, painful and unpleasant, a Likert scale from 0 ('not at all') to 9 ('extremely') was used.

2.4.4. Subjective ratings of the word stimuli

To ensure that the emotional words were experienced as negative and arousing, participants rated each word on the dimensions of valence and arousal using the SAM (Hodes et al., 1985). The SAM is a nonverbal self-report measure composed of five pictograms, depicting increasing levels of valence and arousal. The range is from 1 ("negative" respectively "not arousing") through 9 ("positive" respectively "highly arousing").

2.4.5. Questionnaires

To examine possible differences between the experimental groups in personality variables that may have affected responses to the stress manipulation, processing of emotional stimuli or memory performance, participants filled out the trait scale of the State-Trait Anxiety Inventory (STAI-T, Spielberger, 1970), the Anxiety Sensitivity Index (ASI, Peterson and Reiss, 1992), the Beck Depression Inventory (BDI, Beck et al., 1996) and the Positive Affect and Negative Affect Schedule (PANAS, Watson et al., 1988).

2.4.6. Working memory

The digit span subtest of the Wechsler Adult Intelligence Scale-revised (Wechsler, 1981) was assessed to control for possible differences between groups in working memory capacity.

2.5. Experimental design and general procedure

Participants were randomly assigned to the stress group (n=24) or control group (n=27). The experiment consisted of three testing sessions on respectively day 1, day 2 and day 8. The testing sessions took place between 12 pm and 7 pm to reduce the impact of diurnal variation in cortisol level. All sessions took place in a laboratory setting in a closed cubicle (4.9 x 8.2 ft) with a computer screen. Given that sleep is essential for memory consolidation (Walker and Stickgold, 2006), we instructed participants to get enough sleep for all sessions. To allow for controlled saliva collection, participants were asked to refrain from caffeine, alcohol and excessive exercise from twelve hours before the experiment and to not consume food or drinks (except for water), chew gum, smoke cigarettes or brush their teeth from two hours prior to testing (session 2 and session 3).

Day 1. Participants were informed about the nature and general procedure of the experiment and asked to sign the informed consent form. Participants were told that they were participating in a larger project consisting of several unrelated tasks (i.e., word task, cold pressor challenge, questionnaires and a puzzle task) that were divided over different days to minimize the possibility that performance on the tasks would interfere with each other. We used this cover story to ensure that participants would not study the words outside the experimental context and would not expect the memory test at day 8. Eligibility of the participant was screened using a self-report medical screening, the BDI and the DUDIT. After BP and HR were measured, participants were subjected to the digit span test. Next, the STAI-T, ASI and PANAS were administered. Thereafter, the encoding phase of the declarative memory task was administered.

Day 2. Session 2 started with a 15-min resting period during which participants could read magazines. After the resting period, the first saliva sample (S1) was obtained. Furthermore, BP, HR and the PANAS were assessed. The experimenter started the memory reactivation part of the declarative memory task, which was directly followed by the instructions of the MAST. During the first and third hand immersion trial, BP and HR were assessed. At the end of the MAST a second saliva sample (S2), the PANAS and the subjective stress scales were taken. This was followed by a 20-min resting period during which participants could read magazines again. Ten min after the MAST, a

third saliva sample (S3) was taken and participants filled out the PANAS. Twenty min after the MAST, a fourth saliva sample (S4) was obtained, BP and HR were measured and participants filled out the PANAS.

Day 8. Upon arrival, participants provided a saliva sample (S5) and filled out the PANAS. Next, the experimenter started the final memory test (i.e., cued recall and recognition task). At the end of the experiment, participants were asked about their expectations and motivation during the task and were debriefed about the stress procedure and the surprise memory test.

2.6. Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (SPSS Inc., Chicago, Illinois). Sample characteristics were analysed by one-way analyses of variance (ANOVAs) with Group (Stress versus Control) as between-subject factor. Effect of the stress manipulation was tested by mixed ANOVAs with Time as within-subject factor and Group as between-subject factor. To normalize distributions, PANAS ratings, cortisol concentrations and alpha-amylase were log-transformed. One participant did not provide enough saliva to assay cortisol and alpha-amylase.

Encoding performance was analysed by a mixed ANOVA with word category (war versus disease) and Time (retention test 1 versus retention test 2) as within-subject factor and Group (stress versus control group) as between-subject factor. Memory performance was defined as percentage correct recall on day 8 relative to recall performance on day 1 (second free recall test). To assess differences in memory reactivation and contextualization, memory performance was calculated for each category separately (i.e., reactivated congruent context, reactivated incongruent context, non-reactivated congruent context, non-reactivated incongruent context). No outliers were detected. Memory performance (i.e., percentage correct recall for each category) was analyzed with a mixed-effects ANOVA with the within-subject factors Reactivation and Context and between-subject factor Group.

Furthermore, we explored the contribution of stress responsiveness to memory contextualization. First, we tested whether indicators of stress responsiveness (cortisol, alpha-amylase, HR, systolic BP, diastolic BP and subjective ratings) were correlated with memory contextualization using Pearson's product-moment correlations. In order to obtain a single value of memory contextualization, we subtracted memory performance for the incongruent context from the congruent context. Thus, a larger contextualization index represents more context-dependency of memories. The stress response was calculated for all stress indices and for each participant individually by subtracting the maximum response from baseline response. For the subjective experience of the MAST, we calculated a sum score of the Likert-scale ratings. Indices of stress were screened for possible outliers ($-3.29 < Z < 3.29$). One participant showed a strongly deviating alpha-amylase response ($Z = -5.34$) and one participant showed a strongly deviating cortisol response ($Z = -3.76$). Both data points were discarded. Second, we examined whether stress responsiveness *mediated* memory contextualization by using a multiple mediation model. The advantage of using a multiple mediation model compared to several single mediation models is that such a model can determine to what extent specific variables mediates the effect between the stress manipulation and memory contextualization, conditional on the presence of other mediators in the model (Preacher and Hayes, 2008). While the stress manipulation might trigger a wide range of stress response systems, a multiple mediation model provides the opportunity to determine the specific contribution of each. Preacher and Hayes' (2008) method was followed for assessing multiple mediation, with 5000 bootstrap iterations and 95% bias-corrected confidence intervals, using Mplus version 6.1 (Muthén and Muthén, 2007). Mediators (i.e., cortisol, HR and subjective stress) were centered around the grand mean. Alpha-level was set at .05 for all statistical tests, an alpha-level ≤ 0.08 was considered a trend.

3. Results

3.1. Participant characteristics

Five participants were excluded from further analyses due to either procedural errors ($n=3$), taking medication at session 3 ($n=1$; propranolol) or dizziness during the MAST ($n=1$). The analyses were performed over 46 participants, 22 participants (8 male/14 female) in the stress group and 24 participants (13 male/11 female) in the control group. The stress and control group did not differ on the digit span, ASI, BDI and STAI-T ($F_s < 1.63$, $p_s > .1$). The stress group was however two years older ($M = 22.81 \pm S.E.M. = .70$) than the control group ($M = 20.91 \pm S.E.M. = .38$) ($F(1, 32.63) = 5.60$, $p = .024$, $\eta_p^2 = .12$). Groups did not differ in motivation for the memory tasks ($F_s < 1.0$, $p_s > .1$). None of the participants indicated studying the words outside the experimental context.

3.2. Stress responses to the MAST²

3.2.1. Cortisol concentrations

Exposure to the MAST increased cortisol responses in the stress group (see Table 1). The mixed ANOVA revealed a significant main effect of Group ($F(1.43) = 4.86$, $p = .033$, $\eta_p^2 = .10$), but not of Time ($F < 1.80$), as well as a significant interaction between Time and Group ($F(1.34, 57.61) = 20.47$, $p < .001$, $\eta_p^2 = .32$). Planned comparisons demonstrated that participants in the stress group showed higher cortisol responses 20 and 30 minutes after the start of the stress manipulation in comparison to the control group ($F_s > 12.17$, $p_s < .01$, $\eta_s^2 > .22$). There were no group differences at baseline or directly after the stress manipulation ($F_s < 1.0$). In addition, simple contrasts compared to baseline revealed a difference in slope between the stress and control group at 10, 20 and 30 min after the stress manipulation ($F_s > 10.45$, $p_s < .003$, $\eta_s^2 > .19$).

- Insert Table 1 around here -

3.2.2. Alpha-amylase

² There were no gender differences in response to the MAST, see supplementary results.

Analysis of alpha-amylase showed a marginally significant interaction between Time and Group ($F(2,86)=2.62$, $p=.079$, $\eta_p^2=.06$), in the absence of main effects of Time and Group ($F_s<1.0$). Planned comparisons did not reveal a difference between groups at any time point ($F_s<2.11$). However, simple contrasts compared to baseline showed a significant difference between groups in slope at 20 min after the start of the stress manipulation ($F(1,43)=5.17$, $p=.028$, $\eta_p^2=.11$).

3.2.3. Systolic BP

As expected, the MAST resulted in a strong increase in systolic BP compared to the control task (see Table 1). Repeated measures ANOVAs yielded a significant main effect of Time ($F(2.94,129.17)=20.57$, $p<.001$, $\eta_p^2=.32$) and Group ($F(1,44)=29.01$, $p<.001$, $\eta_p^2=.38$) as well as a significant Time by Group interaction ($F(2.94,129.17)=29.60$, $p<.001$, $\eta_p^2=.40$). Planned comparisons demonstrated higher systolic BP in the stress group than in the control group at t2, t5 and t10 ($F_s>24.97$, $p_s<.001$, $\eta_{s_p}^2>.36$), but no pre- or post-assessment difference ($F_s<1.0$). Furthermore, simple contrasts compared to baseline confirmed a difference in slope at t2, t5 and t10 ($F_s>23.45$, $p_s<.001$, $\eta_{s_p}^2>.34$).

3.2.4. Diastolic BP

Like systolic BP, diastolic BP increased by exposure to the MAST. Analysis revealed significant main effects of Time ($F(2.83,124.57)=15.79$, $p<.001$, $\eta_p^2=.26$) and Group ($F(1,44)=22.42$, $p<.001$, $\eta_p^2=.34$) and a significant Time by Group interaction ($F(2.83,124.57)=16.19$, $p<.001$, $\eta_p^2=.27$). As can be seen in Table 1 and demonstrated by planned comparisons, participants in the stress group showed higher levels of diastolic BP than participants in the control group during and directly after the MAST ($F_s>18.61$, $p_s<.001$, $\eta_{s_p}^2>.29$). There were no differences between groups at pre- or post-assessment ($F_s<1.0$). In addition, simple contrasts compared to baseline showed a difference in slope at t2, t5 and t10 ($F_s<16.87$, $p_s<.001$, $\eta_{s_p}^2>.27$).

3.2.5. Heart rate

Analysis of HR resulted in a significant main effect of Time ($F(3.02, 132.89) = 6.34, p < .001, \eta_p^2 = .13$) and a significant interaction between Time and Group ($F(3.02, 132.89) = 3.71, p = .013, \eta_p^2 = .08$), in the absence of a main effect of Group ($F < 1.0$). Planned comparisons could not reveal a difference between groups at any time point ($F_s < 1.89, p_s > .1$) (see Table 1). However, simple contrasts compared to baseline revealed a significant difference between groups in slope from baseline to t2 exposure ($F(1, 44) = 7.77, p = .01, \eta_p^2 = .15$) and a marginally significant difference in slope from baseline to t5 ($F(1, 44) = 3.92, p = .054, \eta_p^2 = .08$),

3.2.6. Negative Affect

The MAST was successful in inducing negative affect. A mixed ANOVA of the negative affect scale of the PANAS yielded a significant main effect of time ($F(2.10, 92.25) = 9.87, p < .001, \eta_p^2 = .18$) and a significant interaction of Time and Group ($F(2.10, 92.25) = 11.36, p < .001, \eta_p^2 = .21$), in the absence of a main effect of Group ($F < 1.44$). As expected, participants in the stress group showed significantly higher negative affect ratings directly after the MAST than participants in the control group ($F(1, 44) = 6.44, p = .015, \eta_p^2 = .13$). Furthermore, the groups trended towards a difference at baseline ($F(1, 44) = 3.74, p = .06, \eta_p^2 = .08$), with participants in the stress group showing lower negative affect ratings at baseline than participants in the control group. There were no significant differences between groups at post stress assessments ($F_s < 2.69, p_s > .1$). In addition, simple contrasts compared to baseline revealed significant differences in slopes between the groups at every time point ($F_s > 7.47, p_s < .009, \eta_{sp}^2 > .14$) (see Table 1).

3.2.7. Subjective ratings

As expected, participants in the stress group experienced the MAST as more unpleasant, painful and stressful than participants in the control group ($t_s > 5.54, p_s < .001, d_s > 1.65$) (see Table 1).

3.3. SAM Ratings

In contrast to our expectations and the data of Moors et al. (2012), participants rated the words from the two categories slightly differently on the valence and arousal scale of the SAM during encoding.

The war-related words were rated as somewhat more negative and arousing (Valence: $M=3.12$, $SD=.61$; Arousing: $M=5.19$, $SD=1.34$) than the disease-related words (Valence: $M=3.30$, $SD=.63$; Arousing: $M=4.51$, $SD=1.19$) ($F_s > 7.83$, $p < .008$, $\eta_p^2 > .15$). There was no difference in SAM ratings between the stress group and the control group ($F_s < 1.0$).

3.4. Memory Performance

3.4.1. Encoding

Participants memorized on average 29 out of 42 words (range: 18 – 38). The mixed ANOVA revealed significant main effects of Time ($F(1,44)=212.74$, $p < .001$, $\eta_p^2 = .83$) and Category ($F(1,44)=57.44$, $p < .001$, $\eta_p^2 = .57$), in the absence of a significant main effect of Group ($F(1,44)=2.97$, $p = .09$). There were no significant interactions between Time, Category and Group ($F_s < 1.54$). As expected, there was an increase in recall performance from the first to the second retention test. Remarkably, participants learned slightly more disease-related words ($M=15.41$, $SD=2.83$) than war-related words ($M=13.41$, $SD=3.19$). This latter finding together with the SAM ratings seems to contradict previous studies showing a positive relation between recall performance and stronger emotionality of the words (Kensinger and Corkin, 2004). However, both word categories were rated as negative. Possibly, disease related words were more easily personalized than war related words and therefore better recalled.

3.4.2. Cued Recall

To control for individual differences in learning performance, we assessed cued recall performance relative to initial encoding (i.e., percentage correct recall on day 8 relative to recall performance on

day 1 for each category)³. The mixed ANOVA showed only a significant main effect of Context ($F(1,44)=31.06, p<.001, \eta_p^2=.41$). There was no significant main effect of Reactivation or Group ($Fs<1.0$). The expected interaction between Context, Reactivation and Group was not significant ($F<2.44$) and neither were any of the other interactions ($Fs<1.10$). Overall, participants recalled more words in the congruent context than in the incongruent context.

- Insert Figure 3 around here -

Next, we re-analyzed the data with age of the participant as covariate. We consider an analysis of covariance (ANCOVA) appropriate here, given that we randomly assigned participants to condition. Thus, entering age as covariate in the analysis would only remove noise variance from group and not something substantive about group (Miller and Chapman, 2001). The mixed ANCOVA revealed no significant main effects of Context, Reactivation or Group ($Fs<2.0$). Furthermore, there was a marginally significant interaction between Context, Reactivation and Group ($F(1,43)=3.85, p=.056, \eta_p^2=.08$). Follow-up analyses for the reactivated words revealed a marginally significant effect of Context ($F(1,43)=3.47, p=.07, \eta_p^2=.08$), but not Group ($F<1.0$) and an interaction between Context and Group ($F(1,43)=4.02, p=.05, \eta_p^2=.09$) (see Figure 3). Planned comparisons confirmed a Context effect in the stress group ($F(1,43)=19.83, p<.001, \eta_p^2=.32$), but not in the control group ($F<2.79$). Follow-up analyses for the non-reactivated words revealed no significant main or interaction effects of Context and Group ($Fs<1.0$). Critically, to attribute the group difference to post-reactivation stress, a difference between the reactivated and non-reactivated word list is necessary. Yet, follow-up analyses for the stress group and control group separately did not reveal a significant difference between the reactivated and non-reactivated word list, neither in the stress group (Reactivation x Context: $F<1.0$) nor in the control group (Reactivation x Context: $F<3.03$).

³ See supplementary results for the mixed ANOVA with the absolute amount of recalled words on day 8 as dependent variable.

To conclude, the results demonstrate an overall effect of context, indicating that participants more easily recalled words in the original, congruent encoding context than in the incongruent context. After controlling for age differences, we showed that the context-dependency of the reactivated words was stronger in the stress group than in the control group. However, the absence of a difference between the reactivated and non-reactivated words within groups indicates that the selective reactivation procedure may not have fully succeeded (note that the current data cannot provide direct evidence for or against successful *selective* reactivation).

3.5. Cortisol response as mediator of memory contextualization

Correlations. Given that the selectivity of the reminder procedure is doubtful, we examined whether stress responses correlated with memory contextualization overall (i.e., for words from the reactivated and non-reactivated list). As can be seen in Table 2, memory contextualization was related to cortisol response, systolic and diastolic BP. Increase in physiological stress was related to stronger context-dependency of the words (i.e., larger difference between memory recall in the congruent context versus the incongruent context). Note that memory contextualization of the reactivated and non-reactivated words separately showed similar results.

- Insert Table 2 around here –

Next, we examined whether stress reactivity also *mediated* the context-dependency of memory. We performed a multiple mediation analysis with group as independent variable (stress=1; no-stress=-1) and memory contextualization (overall) as dependent variable. Indices of the three major stress response systems were included as mediator, that is increase in cortisol (i.e., index of glucocorticoid activity), increase in heart rate (i.e., index of sympathetic arousal) and the subjective experience of stress (i.e., indicator of psychological stress). As shown in Figure 4, group predicted subjective stress, cortisol and HR response (path a1, a2 and a3). Adjusting for group, there was a

marginally significant relation between cortisol and context-dependency of memory ($p=.07$), but not for subjective stress or HR ($ps>.1$). Importantly, even though there was no direct relation between group and memory contextualization, the cortisol response uniquely mediated the effect of the stress manipulation on memory contextualization ($b=4.38$, 95% CI: 0.78 – 9.66). Heart rate and subjective experience of stress did not mediate between the stress manipulation and memory contextualization. Thus, post-reactivation increase in cortisol resulted in more dependency of contextual information at memory recall.

- Insert Figure 4 -

4. Discussion

The aim of the current study was to examine whether stress during the reconsolidation window affects the contextualization of emotional declarative memories. There are two main findings: (1) post-reactivation stress did not *selectively* affect the context-dependency of reactivated words relative to non-reactivated words, (2) cortisol response mediated the effect of the post-reactivation stress manipulation on memory contextualization for all words (both reactivated and non-reactivated). This latter result indicates that the stronger the post-reactivation cortisol response, the more the recall performance of the study words was dependent on the contextual embedding of these words. The theoretical and clinical implications of the current findings are discussed below.

Remarkably, we found no within-subject differences between the reactivated and non-reactivated words in either the stress group or the control group. Hence the stress manipulation might not have *selectively* affected the reactivated memory. This finding suggests that either the selective reactivation procedure or the creation of two distinct memories was unsuccessful. One possibility is that the spatial context was critically involved in encoding as well as retrieval of the study material. We used two separate emotional categories and a related background picture in order to facilitate the creation of two to be memorized context-specific categories. At test,

participants in both groups recalled more words in the congruent context than in the incongruent context indicating that the word-context (i.e., background picture) association was successful. Nevertheless, the spatial context of the lab setting could have served as a common (meta-)context for the participants, connecting both categories into one memory representation. Moreover, the reminder procedure was carried out in the same spatial context as encoding. The combination of our reminder procedure and the spatial context could have reactivated the memory representation of both word categories instead of only the intended word category. A previous study in humans demonstrated that a spatial context in itself could already be sufficient to reactivate a declarative memory and open-up the reconsolidation window (Hupbach et al., 2008). However, several fear conditioning studies on reconsolidation were successful in selective reactivation, while the distinct fear memory associations were acquired in the same spatial context (Soeter and Kindt, 2011, 2012b). It bears mentioning however that traditional fear conditioning involves rather simple associations between a neutral stimulus and an aversive outcome. Thus, each stimulus has a unique predictive value for the occurrence of the aversive outcome and the distinct fear associations may be less strongly connected through the spatial context than the two word-categories in the current study. It is important to note that based on the current data we cannot provide evidence for whether the *selective* reactivation procedure failed or not. Nevertheless, this post-hoc explanation seems likely given the current findings.

Critically, one might suggest that our reminder procedure failed to open-up the reconsolidation window. A notable shortcoming of the present study design is that we did not incorporate a post-reactivation short-term memory test (PR-STM), whereas reconsolidation is a time-dependent process and the effects on memory reconsolidation should therefore not be observed at PR-STM test. Hence, the stress exposure following memory reactivation could have directly affected memory contextualization instead of affecting the process of memory reconsolidation. However, other memory processes, like retrieval or consolidation, cannot easily explain the finding that cortisol response mediated the effect of the post-reactivation stress manipulation on the context-

dependency of declarative memory because we induced stress after memory reactivation. Still, given that we assume that the selective reminder procedure failed, we cannot rule out that stress exposure enhanced memory contextualization in a non-specific way rather than by affecting reconsolidation. For future research it is essential to incorporate additional control conditions (PR-STM condition, successful within-subject comparison or a between-subject design) to rule out these alternative explanations.

The second main finding is that stress-induced cortisol response after memory reactivation mediated the context-dependency of recall. We induced stress experimentally by exposing participants consecutively to cold pressor tests and arithmetic challenges. The advantage of using an experimental stress manipulation is that it mimics a real-life stress experience and elicits a wide range of physiological and psychological reactivity. Indeed, stress exposure activated the ANS, HPA axis and induced psychological distress. A mediation analysis provides the opportunity to clarify the specific contribution of each stress response system to the effect of a post-reactivation stress manipulation on memory contextualization (Preacher and Hayes, 2008). Furthermore, mediation analysis may even be preferred over group analysis (Kosslyn et al., 2002) when large inter-individual variability is expected, such as in psychophysiological stress responses (Kudielka et al., 2009).

Cortisol response uniquely contributed to the relation between post-reactivation stress and memory contextualization, whereas other parameters of stress such as HR increase and subjective stress did not. The critical role of cortisol is in line with the literature that strongly emphasizes the effects of glucocorticoids on learning and memory processes in general (Joëls and Baram, 2009) and memory contextualization in specific (van Ast et al., 2013; van Ast et al., 2014). A previous study showed that cortisol administration three hours before encoding enhanced emotional memory contextualization, whereas cortisol administration 30 min before encoding impaired contextualization in healthy participants (van Ast et al., 2013). The current findings extend our knowledge on contextualization of emotional declarative memory by showing that stress-induced cortisol can also enhance the context-dependency after memory reactivation. Even though it remains

speculative, it seems likely that the hippocampus mediated the current observations. The critical role of the hippocampus in changes in context-specificity during memory reconsolidation is suggested by an earlier study in rats (Winocur et al., 2009). Winocur and colleagues (2009) tested the effect of hippocampal lesions during the reconsolidation window on remote contextual fear memories. They showed that only when the fear acquisition context served as a reminder, the fear memory regained its context-specificity and became susceptible to hippocampal lesions. In contrast, exposure to a different context only reactivated general features of the remote contextual fear memory, which was less affected by hippocampal lesions.

Our findings indicate that a stress-induced cortisol response enhances the context-specificity of emotional memory and suggest that post-reactivation cortisol can prevent memory generalization. Over time, memory transfers from specific to more general and gist-like memory representations (Winocur et al., 2009; Nadel and Hardt, 2010). This transformation is to a certain degree observed in the control group, which showed less context-dependency for the reactivated words compared to the stress group. The suggestion that post-reactivation stress prevents memory to generalize over contexts seems to be at odd with the idea that stress facilitates gist processing (Payne et al., 2002), for example in patients with PTSD (Ehlers and Clark, 2000; Liberzon and Sripada, 2007). This apparent discrepancy in cortisol effects may partly be explained by differences in memory reactivation. In the current study, we reactivated the memory of the study words by presenting participants a contextual reminder (background picture) in the encoding context, which may have triggered context-specific memory (Winocur et al., 2009). In contrast, retrieval of trauma memory in patients with PTSD is characterized by re-experiencing gist-like aspects of the trauma memory, which lack temporal and spatial context (Ehlers and Clark, 2000). Thus, when retrieval of trauma memory is accompanied by post-retrieval stress, generalization rather than context-specificity of the memory may be facilitated. In addition, it has been suggested that patients with PTSD may predominantly process the sensory impressions and perceptual characteristics of the event (i.e., data-driven processing) rather than the meaning and context of the event (i.e., conceptual-driven processing, Ehlers and Clark, 2000; Kindt et

al., 2008). Following this line of reasoning, stress-induced cortisol after memory reactivation may have facilitated conceptual-driven processing during reactivation (i.e., attention to contextual information).

Alternatively, post-reactivation stress effects may follow an inverted U-shape curve (Marin et al., 2011), like stress effects on memory consolidation (Abercrombie et al., 2003). Strong elevations of cortisol after reactivation have been shown to disrupt memory performance, whereas weak cortisol elevations enhanced memory performance (Marin et al., 2011). But it is still unclear whether post-reactivation stress effects on memory contextualization follow a similar pattern.

A few limitations of the current study should be mentioned. First, we used words that were related to two emotional categories. During encoding on day 1, the disease-related words were better recalled than the war-related words (see supplementary results). Even though we corrected for initial recall performance by using percentage scores and counterbalancing the *selective* reactivation procedure, this encoding difference may have affected the results. Second, we did not incorporate neutral control words. Previous studies have shown that stress exposure and stress hormones typically affect memory performance for emotional stimuli (e.g., Cahill et al., 2003; McGaugh, 2004) as a result of the interaction between emotion-induced arousal by the study material and stress hormones (Roosendaal et al., 2009). The current design did not allow us to test this interaction. Third, participants in the stress group showed somewhat lower negative affect scores than participants in the control group prior to memory reactivation and the stress manipulation. This might indicate that participants in the control group showed more anticipation to the stress manipulation compared to the stress group. We cannot rule out that anticipation stress in the control group may have affected memory reactivation or the control manipulation. Fourth, we included both male and female participants in the current experimental design. It is well-known that male and female participants respond differently to stress manipulations (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005; Cornelisse et al., 2011). Given the relatively small sample size, the

current design did not allow for an appropriate examination of gender differences in the effect of stress exposure on memory reconsolidation and memory contextualization.

To conclude, the current results demonstrate that stress-induced cortisol strengthens the context-dependency of emotional declarative memories. This finding suggests that moderate levels of post-reactivation stress might serve an adaptive function against generalization of emotional memories over contexts.

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Tables

	Stress n=22	Control n=24
Systolic BP		
<i>Day 1</i>		
	123.26 ± 2.12	120.28 ± 2.66
<i>Day 2</i>		
t-5	116.27 ± 2.18	113.29 ± 2.17
t2	135.68 ± 3.32**	112.88 ± 2.17
t5	140.00 ± 3.18**	109.29 ± 1.95
t10	132.36 ± 3.88**	110.50 ± 2.21
t30	114.27 ± 2.18	112.17 ± 2.21
<i>Day 8</i>		
	119.75 ± 2.40	118.60 ± 3.22
Diastolic BP		
<i>Day 1</i>		
	74.34 ± 1.66	73.88 ± 1.72
<i>Day 2</i>		
t-5	71.14 ± 2.10	69.46 ± 1.39
t2	87.86 ± 2.62**	70.38 ± 1.49
t5	89.32 ± 3.00**	68.92 ± 1.32
t10	81.77 ± 2.62**	69.00 ± 1.51
t30	73.09 ± 2.81	69.88 ± 1.89
<i>Day 8</i>		
	70.50 ± 2.00	70.73 ± 2.18
Heart Rate		
<i>Day 1</i>		
	67.83 ± 2.22	71.00 ± 2.01
<i>Day 2</i>		
t-5	66.09 ± 1.88	69.29 ± 2.71
t2	72.86 ± 2.56	68.50 ± 1.93
t5	69.50 ± 2.22	68.17 ± 1.79
t10	66.09 ± 2.35	68.63 ± 1.69
t30	62.14 ± 2.47	66.38 ± 2.01
<i>Day 8</i>		
	64.92 ± 2.00	69.28 ± 2.35

Cortisol		
Day 2		
t-5	10.21 ± 0.69	11.81 ± 1.27
t10	10.76 ± 0.71	10.29 ± 1.03
t20	16.61 ± 2.22**	9.37 ± 1.02
t30	16.25 ± 2.39**	8.76 ± 1.00
Alpha-Amylase		
Day 2		
t-5	196.50 ± 28.89	194.98 ± 21.74
t10	250.87 ± 44.74	198.00 ± 22.08
t20	252.69 ± 39.19	189.70 ± 27.50
Negative Affect		
Day 1		
	1.37 ± 0.10	1.46 ± 0.14
Day 2		
t-5	1.19 ± 0.05 [#]	1.40 ± 0.09
t10	1.73 ± 0.17*	1.25 ± 0.06
t20	1.38 ± 0.10	1.18 ± 0.04
t30	1.28 ± 0.08	1.12 ± 0.03
Day 8		
	1.34 ± 0.09	1.27 ± 0.07
Subjective Stress		
Unpleasant	7.41 ± 0.40**	3.04 ± 0.40
Painful	6.14 ± 0.52**	0.17 ± 0.10
Stressful	4.91 ± 0.60**	1.00 ± 0.37

Table 1 Effects of psychosocial stress exposure on systolic and diastolic blood pressure (BP; in mmHg), heart rate (HR, in beat per minute), salivary cortisol, salivary alpha-amylase and negative affect ratings assessed before (t-5), during (t2 and t5) and after (t10, t20, t30) the stress manipulation in the two experimental groups. Subjective experience of stress was assessed directly after stress exposure (t10).

Means ± S.E.M.; [#] $p < .08$, * $p < .05$, ** $p < .01$.

	1	2	3	4	5	6	7	8	9	10
1. Contextualization – Reactivated										
2. Contextualization – Non-reactivated	.587**									
3. Contextualization - General	.901**	.876**								
4. Subjective Stress	.265	.041	.175							
5. ΔNegative Affect	.139	-.003	.082	.619**						
6. Δcortisol	.345*	.277 [#]	.362*	.669**	.428**					
7. ΔsAA	.152	.062	.119	.390**	.296 [#]	.231				
8. ΔsysBP	.298*	.193	.282 [#]	.649**	.383**	.520**	.338*			
9. ΔdiaBP	.397**	.171	.323*	.596**	.272 [#]	.566**	.476**	.766**		
10. ΔHR	.252	.140	.231	.439**	.211	.453**	.275 [#]	.449**	.392**	

Table 2 Pearson correlations between stress reactivity and contextual dependency of memories.

Note: Δ means peak level minus baseline level; [#] $p < .08$; * $p < .05$; ** $p < .01$

Figures

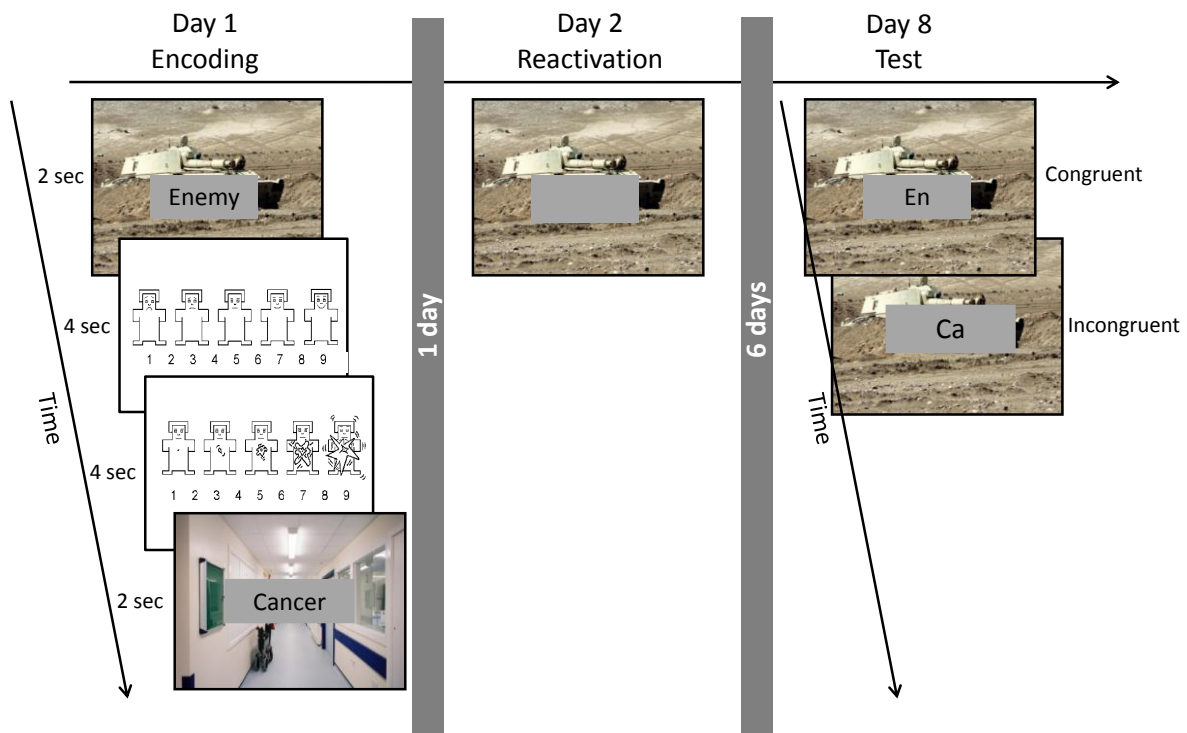


Figure 1 Overview of the experimental design.

Day 1 – Memory encoding, Day 2 – Memory reactivation, Day 8 – Memory test

On day 1, participants were shown 24 war-related and 24 disease-related words against a content-related background picture (i.e., tank in war scene or hospital corridor). As shown in the figure, participants rated the words on a valence and arousal scale after each trial. At the second presentation of the words, participants were instructed to learn the words. The participants were exposed to a free recall task at the first as well as the second encoding task. On day 2, participants were reminded of one word list. After a week on day 8, memory performance was assessed with a cued recall task. Half of the word stems were presented in the encoding context (congruent condition) and half of the words were presented in the other context (incongruent). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

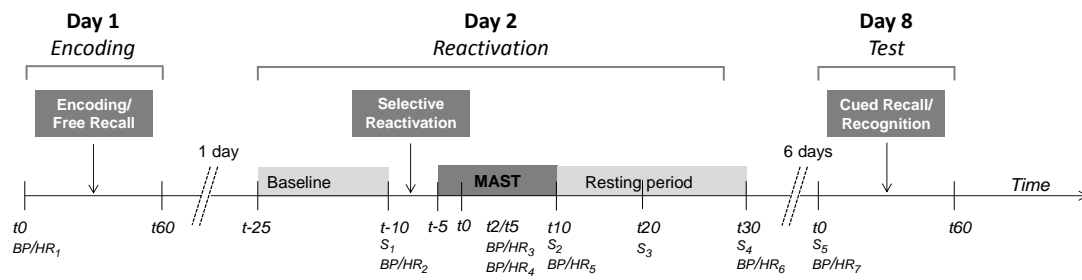


Figure 2 Timeline depicting the saliva, blood pressure and heart rate assessments.

Blood pressure (BP) and heart rate (HR) were assessed at seven time points throughout the experiment: at baseline of each session and 2, 5, 10 and 30 min after the start of the stress manipulation (BP/HR₁ – BP/HR₇). Saliva samples (S₁ – S₅) were taken five times: 5 min before and 10, 20 and 30 min after the start of the stress manipulation and one sample at the start of the test session on day 8.

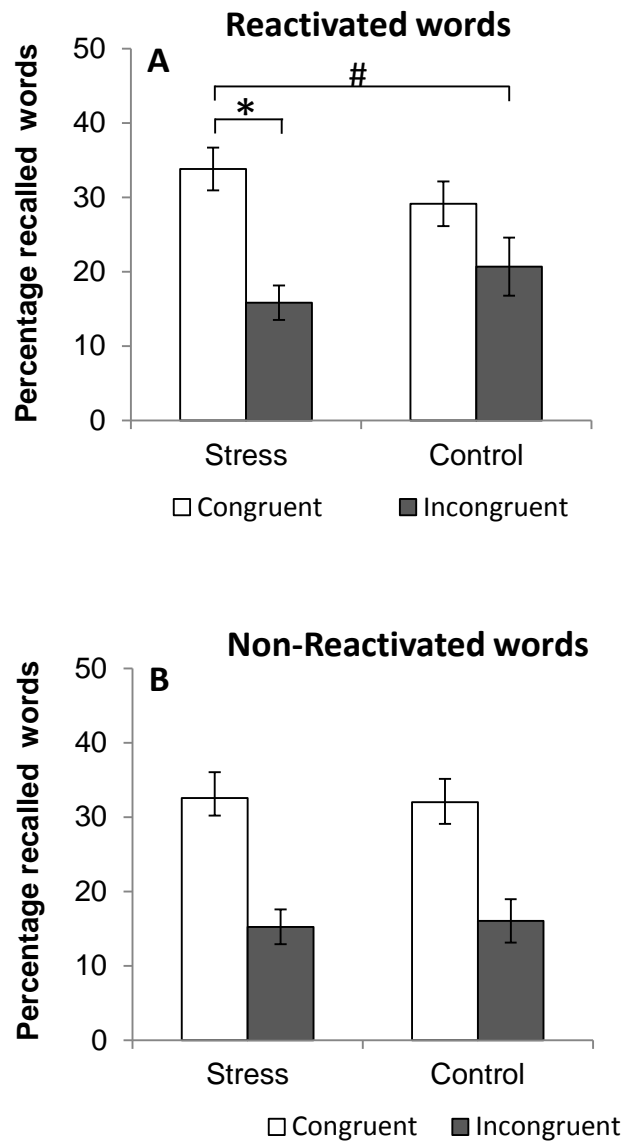


Figure 3 Memory performance (**day 8**)

Memory performance indexed by percentage recalled words at test relative to performance on day 1 as a function of Context. Panel A depicts memory performance for the reactivated words. Panel B depicts memory performance for the non-reactivated words. Controlled for age, the stress group showed somewhat more contextual dependency of reactivated words than the control group.

$p < .08$; * $p < .05$; Error bars represent S.E.M.

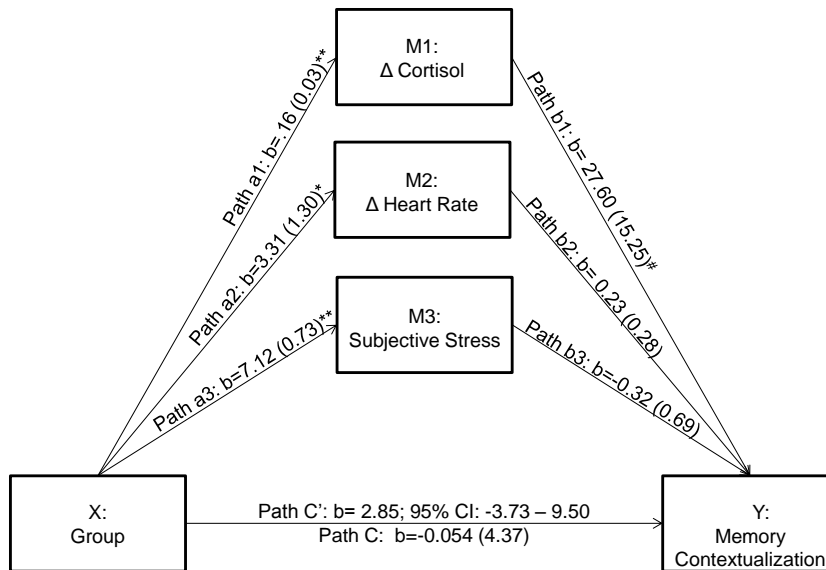


Figure 4 Multiple mediation model of the relation between group (Stress and Control Group) and memory contextualization (i.e., recall performance for the congruent context (reactivated and non-reactivated words) minus recall performance for the incongruent context (reactivated and non-reactivated words) with increase in cortisol, heart rate (HR) and subjective stress as mediators. The point estimates (S.E.M) are presented in the figure.

$p < .08$; * $p < .05$; ** $p < .01$